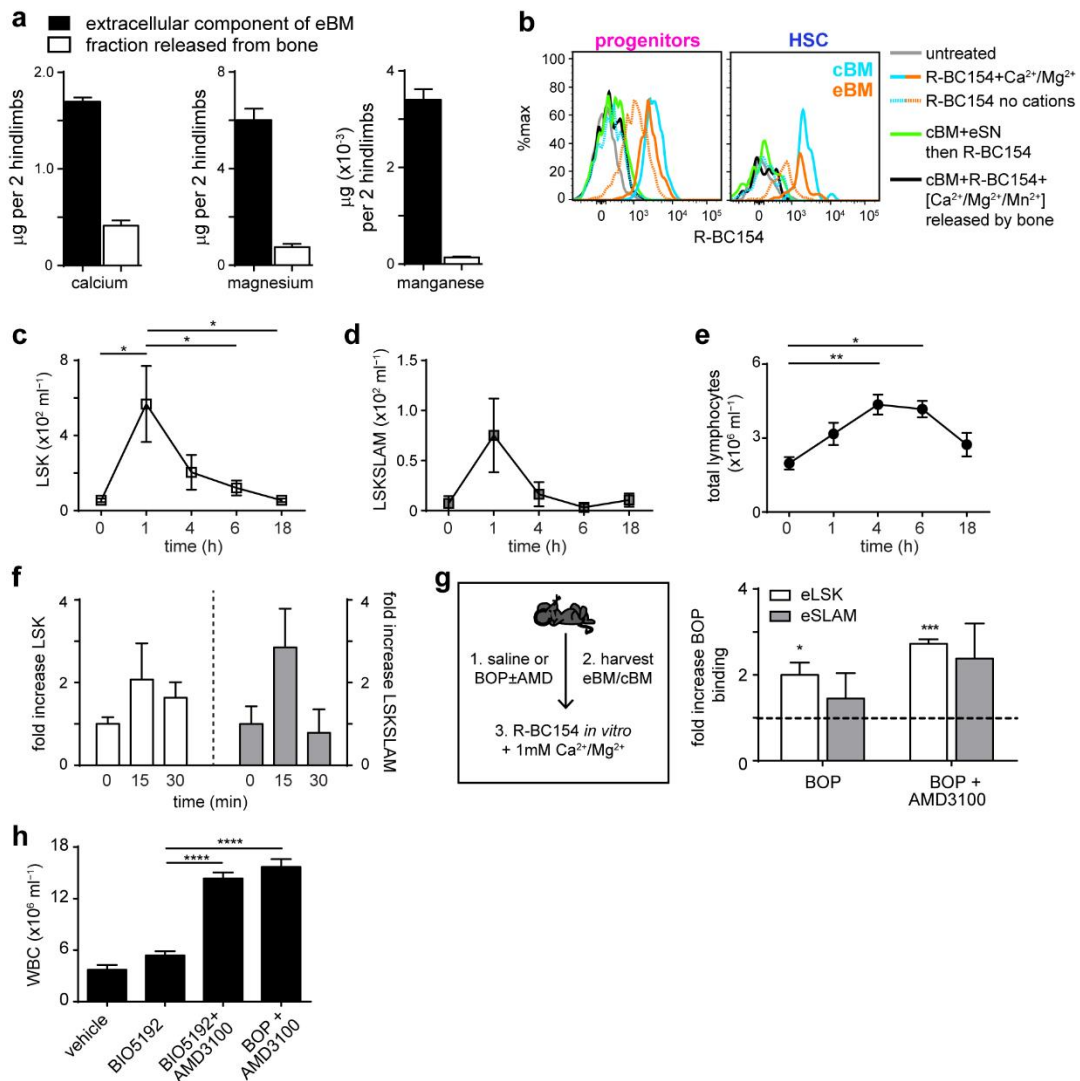


Supplementary Figure 1.

(a) R-BC154 specifically binds murine α_4 and α_9 expressed by CHO cells in the presence of divalent metal cations ($\text{Ca}^{2+}/\text{Mg}^{2+}$) (red line). R-BC154 binding is abrogated in the presence of EDTA (black line). (b) Generation of humanized NODSCIDIL2R $\gamma^{-/-}$ (huNSG) mice from cord blood (CB) mononuclear cells (MNC) and flow cytometric analysis of BM white blood cell (WBC) CD34 $^+$ CD38 $^+$ progenitors, CD34 $^+$ CD38 $^+$ HSC and CD34 $^-$ CD38 $^+$ committed cells. (c) Expression of α_4 (solid line) on huNSG BM committed cells, progenitors and HSC. Isotype = dashed line. (d) Quantified data from Fig. 1k depicting R-BC154 binding in the presence of $\text{Ca}^{2+}/\text{Mg}^{2+}$ to huNSG BM committed cells (CC), progenitors (prog) and HSC. n=3. (e) Positive correlation of $\alpha_9\beta_1$ expression versus R-BC154 binding to $\alpha_9\beta_1$ ($P<0.0001$). P -value represents statistically significant non-zero slope using linear regression analysis. Each circle depicts a single CB CD34 $^+$ CD38 $^-$ cell and data is representative of 3 individual CB samples. (f) Dot plot of murine BM progenitors (Lin $^-$ Sca-1 $^+$ c-kit $^+$; LSK) and HSC (LSKCD150 $^+$ CD48 $^-$; LSKSLAM). (g) R-BC154 binding to murine progenitors and HSC in the presence of $\text{Ca}^{2+}/\text{Mg}^{2+}$ (red bar) or EDTA (black bar). n=3. Representative of at least 3 independent experiments. (h) R-BC154 binding in the presence of $\text{Ca}^{2+}/\text{Mg}^{2+}$ to WT and α_4 and α_9 knockout (KO) BM murine progenitors and HSC. n=3. (i) Representative comparison of *in vitro* staining of R-BC154 in the presence of exogenous $\text{Ca}^{2+}/\text{Mg}^{2+}$ and *in vivo* administration of R-BC154. n=3. (j) Schematic of assessment of the specificity and reversibility of R-BC154 binding *in vivo*. (k) Displacement of R-BC154 binding to BM progenitors and HSC *in situ* using exogenous competing BOP *in vitro*. n=3. (l) Expression of α_4 and $\alpha_9\beta_1$ (solid line, dashed line IgG1 isotype controls) on central (cBM) and endosteal (eBM) progenitors and HSC from huNSG BM. 7-8 week old female NSG mice were transplanted with CB CD34 $^+$ cells (b,c,d,l). 7-8 week old female mice were used for (f,g). 6-12 week old female and male mice were used for (h). 8 week old female mice were used for (i-k). All data is mean \pm s.e.m. Analysis using one-way ANOVA (d,k) or student's t-test (g,h), *** $P<0.005$, **** $P<0.001$.



Supplementary Figure 2.

(a) Total calcium, magnesium and manganese content in the extracellular component of endosteal BM (eBM) compared to the amount mechanically released from bone during endosteal cell isolation. (b) Analysis of R-BC154 binding to cBM progenitors (LSK) and HSC (LSKSLAM) when pre-treated with eBM supernatant (eSN) or with exogenous Ca²⁺/Mg²⁺/Mn²⁺ at concentrations determined in (a). R-BC154 binding to eBM and cBM LSK and LSKSLAM in the presence of 1 mM Ca²⁺/Mg²⁺ or in the absence of cations were used as controls. Data is representative of 4 samples. (c) Extended time-course analysis of total LSK, (d) LSKSLAM and (e) lymphocyte content in PB for up to 18 hours after a single dose of BOP. n=5. (f) Analysis of LSK and LSKSLAM content in PB of mice treated with saline or R-BC154 over 30 minutes. Data is a pool of 2 independent experiments. n>6. (g) Analysis of *in vivo* BOP binding to endosteal versus central LSK and LSKSLAM following the administration of BOP alone or BOP in combination with AMD3100. Data is presented as fold increase in BOP binding to endosteal cells relative to their central counterparts (dotted line). n=3. *P*-values represent statistically significant results using paired t-test between central and endosteal populations within each treatment group. (h) PB white blood cell (WBC) content 1 hour after a single dose of vehicle (n=5), BIO5192 alone (n=5) or in combination with AMD3100 (n=5), or BOP in combination with AMD3100 (n=3). 7-8 week old female mice were used for (a-g). 7-8 week old male mice were used for (h). All data is mean±s.e.m. Analysis using one-way ANOVA (c,d,e,f,h), **P*<0.05, ***P*<0.01, ****P*<0.005, *****P*<0.001.

Supplementary Table 1. Anti-mouse antibodies

Antibody	Clone	Isotype	Cat #	Concentration
CD3e	17A2	rat IgG2b	100234	1 µg ml ⁻¹
CD4, L3T4	GK1.5	rat IgG2b	553729	1 µg ml ⁻¹
CD8a, Ly-2	53-6.7	rat IgG2a	553031	1 µg ml ⁻¹
CD11b, Mac-1	M1/70	rat IgG2b	101218	0.25 µg ml ⁻¹
CD45	30-F11	rat IgG2b	564279	1 µg ml ⁻¹
CD45R, B220	RA3_6B2	rat IgG2a	563103 103226	1 µg ml ⁻¹ 0.5 µg ml ⁻¹
CD48	HM48-1	A. hamster IgG1	103404	1 µg ml ⁻¹
CD117, c-kit	2B8	rat IgG2b	564011	1 µg ml ⁻¹
CD150 (SLAMF1)	TC15- 12F12.2	rat IgG2a	115931	1 µg ml ⁻¹
GR-1, Ly-6G	RB6-8C5	rat IgG2b	108418	0.25 µg ml ⁻¹
Sca-1, Ly-6A/E	E13- 161.7	rat IgG2a	122514	0.4 µg ml ⁻¹
TER119	TER119	rat IgG2b	560509	0.4 µg ml ⁻¹

Supplementary Table 2. Anti-human antibodies

Antibody	Clone	Isotype	Cat #	Concentration
CD3	UCHT1	Mouse IgG1	555330	5 µg ml ⁻¹
CD11b	ICRF44	Mouse IgG1	555386	5 µg ml ⁻¹
CD14	M5E2	Mouse IgG2a	555396	5 µg ml ⁻¹
CD16	3G8	Mouse IgG1	555404	5 µg ml ⁻¹
CD20	2H7	Mouse IgG2b	555621	5 µg ml ⁻¹
CD24	ML5	Mouse IgG2a	555426	5 µg ml ⁻¹
CD235a	GA-R2	Mouse IgG2b	555569	5 µg ml ⁻¹
CD34	8G12	Mouse IgG1	348053	5 µg ml ⁻¹
CD38	HIT2	Mouse IgG1	562444 347687	2.5 µl per test 0.6 µg ml ⁻¹
CD45	HI30	Mouse IgG1	563717	0.5 µl per test
CD49d (α₄)	9F10	Mouse IgG1	304314	1 µl per test
α₉β₁	Y9A2	Mouse IgG1	MAB2078Z	20 µg ml ⁻¹